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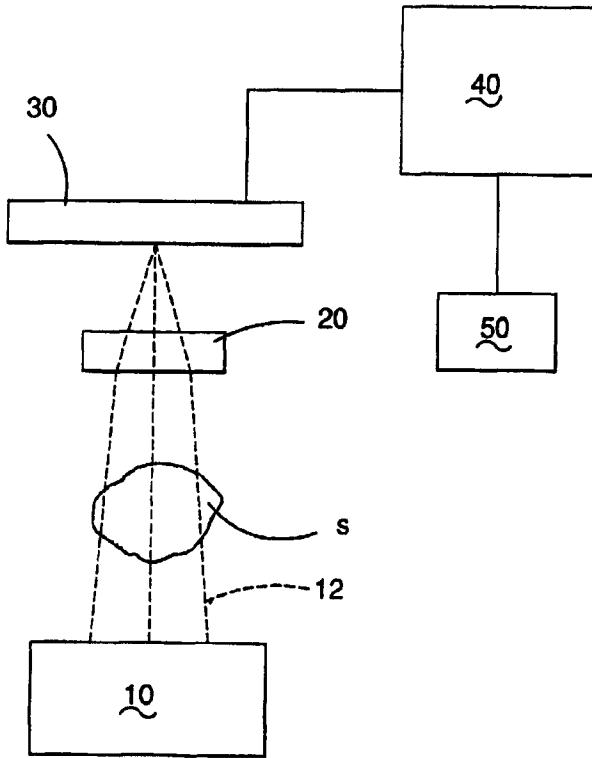
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(54) Title: PHASE TECHNIQUE FOR DETERMINING THICKNESS, VOLUME AND REFRACTIVE INDEX



(57) Abstract: Method and apparatus for determining a parameter of a sample (S) such as a cell is disclosed. The method and apparatus detect phase data relating to the sample by detecting light reflected or transmitted through the sample (S) by a CCD array (30). Processor (40) manipulates the phase data with other known characteristics of the sample to determine the required parameter.

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## PHASE TECHNIQUE FOR DETERMINING THICKNESS, VOLUME AND REFRACTIVE INDEX

Field of the Invention

5      This invention relates to a method and apparatus for determining a parameter of a sample and, in particular, the volume or refractive index of a biological sample such as a cell.

10     The volume measured, in the preferred embodiment of the invention, is the "optical path volume" of the sample. When measuring cell physiology changes under the influence of external effects, measuring the change in optical path volume is just as effective as measuring the actual spatial volume. If the refractive index distribution of the cell 15    or sample is known, the spatial volume of the cell can be determined.

Background of the Invention

20     Phase imaging of objects and, in particular, biological samples, is a useful tool to provide information relating to the object which may not be available in conventional intensity or absorption images.

25     International Patent Application No. PCT/AU99/00949 owned by The University of Melbourne discloses a method and apparatus for producing phase images by solving the transport of intensity equation. In order to solve that equation, images of the object are collected by a charge coupled device. The images comprise an in focus image and 30    preferably two defocused images of the object. The data obtained by the charge coupled devices is processed so as to produce a phase image of the object.

35     The phase image includes phase data relating to the object which is completely independent of intensity or absorption data.

- 2 -

Determining a change in volume of an object such as a cell can be useful in determining the reaction of the cell to various stimuli such as the addition of drugs or other substances. By determining a change in volume an

5 indication can be obtained of the effect of the substance or stimuli on the cell and therefore information relating to possible therapeutic properties of the drug or stimuli or undesirable side effects can be obtained.

10 Obviously, cells are extremely small and it is extremely difficult to therefore obtain an indication of any change in volume or the specific volume of the cell before or after a particular procedure.

15 Furthermore, obtaining information relating to refractive index can also provide valuable information relating to the cell and, in particular, the substance from which the cell is formed.

20 Summary of the Invention  
The object of the present invention is to provide a method and apparatus for determining a parameter of a sample such as a cell.

25 The invention may be said to reside in a method of determining a parameter of a sample including the steps of:  
detecting phase data of a radiation wave field emanating from the sample;  
combining the phase data with a known parameter

30 of the sample; and  
determining the required parameter from the phase data and the known parameter of the cell.

In one embodiment of the invention, the parameter comprises

35 the volume of the cell and the method includes:  
determining the physical thickness of the sample by multiplying phase data contained in the radiation wave

field by the wavelength of the radiation and dividing by a prescribed factor of the refractive index of the sample, with the refractive index being the known parameter of the sample; and

5 multiplying the physical thickness by the area of the sample to obtain the volume of the sample.

In another embodiment, the parameter is the refractive index of the sample and the method includes:

10 confining the sample to a known thickness;  
detecting the wave field emanating from the sample confined to the known thickness;  
determining phase data contained in the radiation wave field emanating from the sample;

15 multiplying the phase data by the wavelength of the radiation; and  
dividing the phase data multiplied by the wavelength, by a prescribed factor multiplied by the physical thickness to which the sample is confined.

20 The invention may be said to reside in an apparatus for determining a parameter of a sample including the steps of:

detecting means for detecting phase data of a radiation wave field emanating from the sample;  
25 processing means for combining the phase data with a known parameter of the sample; and  
determining the required parameter from the phase data and the known parameter of the cell.

30 In one embodiment of the invention, the parameter comprises the volume of the cell and the processor is for:

determining the physical thickness of the sample by multiplying phase data contained in the radiation wave field by the wavelength of the radiation and dividing by a 35 prescribed factor of the refractive index of the sample, with the refractive index being the known parameter of the sample; and

- 4 -

multipling the physical thickness by the area of the sample to obtain the volume of the sample.

In another embodiment, the parameter is the refractive 5 index of the sample and the apparatus includes:

means for confining the sample to a known thickness;

the processor is for detecting the wave field emanating from the sample confined to the known thickness;

10 determining phase data contained in the radiation wave field emanating from the sample;

multipling the phase data by the wavelength of the radiation; and

15 dividing the phase data multiplied by the wavelength by a prescribed factor and the physical thickness to which the sample is confined.

The invention may be said to reside in a method of determining the volume of a sample including the steps of:

20 determining the physical thickness of the sample by multiplying phase data contained in a beam of light emanating from the sample by the wavelength of the beam of light and dividing by a prescribed factor of the refractive index of the sample; and

25 multipling the physical thickness by the area of the sample to obtain the volume of the sample.

Thus, by collecting the phase data relating to the object and multiplying by known parameters such as the wavelength 30 of the light beam and the refractive index of the object, the physical thickness of the object can be determined which in turn enables the volume to be determined by multiplying the physical thickness by an area value to obtain a volume.

35

Preferably the physical thickness is obtained at each pixel of a charge coupled device which detects the light beam

- 5 -

emanating from the object and the area value is the area value of the pixel.

5 Preferably for all pixels which contain the image of the object, the area of the pixel is multiplied by the physical thickness at each pixel and summed in order to provide the volume of the object.

10 Preferably the step includes mapping on an image of an object the location of the object on the charge coupled device so that all pixels within the mapped area can be determined, determining the thickness of the cell attributed to each of those pixels by multiplying phase data at each pixel by the wavelength of the light and then 15 dividing by  $2\pi$  multiplied by the refractive index of the object, and summing the volume obtained for each of the pixels.

20 The invention may also be said to reside in an apparatus for determining the volume of a sample, including:

means for detecting light emanating from an object so as to enable phase data relating to the object to be determined; and

25 processing means for determining the phase data from the detected light, and for determining the physical thickness of the object by multiplying the phase data by wavelength of the light and dividing by a factor of the refractive index of the light; and

30 then calculating the cell volume by an area value multiplied by the physical thickness.

Preferably the physical thickness is obtained at each pixel of a charge coupled device which detects the light beam emanating from the object and the area value is the area 35 value of the pixel.

Preferably volume is obtained by the sum of the thickness

- 6 -

at all pixels which contain the image of the object.

Preferably the step includes mapping on an image of an object the location of the object on the charge coupled 5 device so that all pixels within the mapped area can be determined, determining the thickness of the cell at each of those pixels by multiplying phase data at each pixel by the wavelength of the light and then dividing by  $2\pi$  multiplied by the refractive index of the object, and 10 summing the volume obtained for each of the pixels.

The invention, in a further aspect, may be said to reside in a method of determining the refractive index of a sample, including the steps of:

15           detecting radiation emanating from the sample;  
              confining the sample to a predetermined thickness;  
              determining phase data relating to the radiation wave field emanating from the sample which is confined to 20 the predetermined thickness;  
              multiplying the phase data by wavelength, and dividing the phase data multiplied by wavelength, by a prescribed factor multiplied by the predetermined thickness to thereby provide the refractive index.

25           Preferably the sample is confined to the known thickness by squeezing the sample between a pair of plates.

Preferably the predetermined factor is  $2\pi$ .

30           The invention, in a further aspect, may be said to reside in an apparatus for determining the refractive index of a sample, including:  
              detector means for detecting radiation emanating 35 from the sample;  
              means for confining the sample to a predetermined thickness;

- 7 -

processing means for determining phase data relating to the radiation wave field emanating from the sample which is confined to the predetermined thickness; and

5 multiplying the phase data by wavelength and dividing the phase data multiplied by wavelength by a prescribed factor multiplied by the predetermined thickness to thereby provide the refractive index.

10 Preferably the means for confining comprises a pair of plates.

Preferably the predetermined factor is  $2\pi$ .

15 Brief Description of the Drawings

A preferred embodiment of the invention will be described, by way of example, with reference to the accompanying drawings in which:

20 Figure 1 is a view of a phase image of an object such as a cell;

Figure 2 is a three dimensional rendering of the phase image of the cell of Figure 1;

Figure 3 is a schematic view of an apparatus embodying the invention;

25 Figure 4 is a side view of a second embodiment of the invention; and

Figure 5 is a plan view of the embodiment of Figure 4.

30 Description of the Preferred Embodiment

Figure 1 shows a phase image of a cell such as a cheek cell which has been produced in accordance with the teachings of the aforementioned International application owned by The University of Melbourne (the contents of which are incorporated into this specification by this reference).

Figure 2 shows a three dimensional rendering of the phase

- 8 -

image of the cheek cell of Figure 1.

Figure 3 is a schematic view of an apparatus embodying the invention for producing the phase image and also for 5 determining the cell volume. The apparatus comprises a light source 10 which supplies light which penetrates through the sample S (ie. the cheek cell) and which is focused by an optical system 20 onto a charge coupled device 30. By manipulating the optical elements 20, an in 10 focus and defocused images of the sample S are produced which provide data enabling the transport of intensity equation to be solved in accordance with the teachings of the aforesaid International application so as to produce phase data to enable the phase image of Figure 1 to be 15 produced by a processor 40.

By viewing the phase image of Figure 1 on a monitor 50, the phase image of the cell can clearly be seen. The outline of the cell or area of the cell can be produced by moving a 20 cursor around the cell so as to produce a trace 60 as shown in Figure 1. This trace 60 will identify all of the pixels of the charge coupled device 20 within the cell area. Each pixel of the charge coupled device within the cell area will have received phase information from the sample cell 25 which can be given in radians. The phase at each pixel is given by the following equation:

$$\text{Phase(radians)} = (2\pi \times \text{optical path length}) + (\text{wavelength})$$

30 The optical path length through the sample cell S is given by the refractive index of the sample cell S times the physical thickness of the cell at each pixel location. Thus, the phase therefore equals;

$$35 (2\pi \times \text{refractive index} \times \text{physical thickness}) + (\text{wavelength})$$

Thus, the physical thickness equals;

- 9 -

(phase x wavelength) + (2π x refractive index)

Since the phase information can be read from the charge  
5 coupled device 30 in radians and a wavelength of the light  
beam 12 produced by the light source 10 is accurately known  
and since the refractive index of the sample cell can  
usually be estimated with a relatively good degree of  
accuracy, the physical thickness of the cell at each pixel  
10 within the area 60 can be determined. The actual cell  
volume can therefore be calculated because the area of each  
pixel is known and the volume will therefore be given by;

15  $\Sigma$  (physical thickness<sub>n</sub>) x (area of individual cell)  
where n is the number of pixels in the area 60.

That is, by multiplying the physical thickness at each  
pixel by the area of each pixel the individual volumes at  
20 each pixel can be determined and those individual volumes  
are then added together to produce the total volume of the  
cell S.

25 Thus, the volume of the cell can be calculated by the  
processor 40.

The preferred embodiment of the invention therefore enables  
the cell volume to be determined so that the volume before  
and after a particular procedure can be determined to  
30 obtain some information as to the reaction to the cell to  
the procedure.

Similarly, if the volume does not change through an  
experiment, the relative refractive index before and after  
35 the experiment can also be obtained. Thus, changes in cell  
volume or changes in refractive index due to a particular  
procedure can be determined.

Furthermore, the ratio of the volume before the procedure to that after the procedure can also be determined by dividing the volume before the procedure with the volume 5 after the procedure. This technique has the advantage that the refractive index is assumed to be the same before and after the procedure it is effectively cancelled out and therefore if any inaccuracy in the actual known refractive index of the sample occurs, that inaccuracy is cancelled 10 out by obtaining the ratio of the volume before to the volume after in accordance with the above equations.

In general, the refractive index of biological samples such 15 as cells can be accurately approximated because the cells are mostly water and, of course, the refractive index of water is well known.

If the refractive index is not accurately known then the ratio of the volume before a procedure to that after a 20 procedure will still provide accurate information as to the change in effective volume caused by the procedure because, as mentioned above, the refractive index will effectively be cancelled out when the before and after ratio are obtained, thereby removing any inaccuracy introduced by the 25 fact that the refractive index is not known.

Figures 4 and 5 show a second embodiment of the invention in which, rather than determine the volume of the sample, the refractive index of the sample is determined. 30 Following from the equations mentioned above, the refractive index of the sample equals:

$$(\text{phase} \times \text{wavelength}) + (2\pi \text{ physical thickness})$$

35 Thus, if the physical thickness is set, then the refractive index can be calculated from that set physical thickness, the phase data relating to the sample, which is determined

- 11 -

in the above-mentioned manner and also the wavelength of the radiation which emanates from the sample.

As shown in Figure 4, in order to define the physical  
5 thickness of the sample, the sample S is confined between  
two thin transparent plates 50 and 60 which are separated  
by a known distance D. That is, the plates 50 and 60 are  
moved together to confine the sample and squeeze the sample  
so that it has a thickness D as shown in Figure 4.

10 When the radiation is transmitted through the sample S (and  
the plates 50 and 60) the radiation can be detected in the  
manner described above so that the phase data relating to  
the radiation wave field emanating from the sample can be  
15 calculated in accordance with the algorithm described  
above.

20 The refractive index of the sample can then therefore be  
calculated by the processor 40 of Figure 2 according to the  
above equation.

Once the refractive index is calculated, the refractive  
index can be used to determine the material from which the  
cell is formed to provide information relating to the  
25 structure and composition of the cell, which is useful in  
identifying the cell and also diagnosis of the condition of  
the cell.

30 Since modifications within the spirit and scope of the  
invention may readily be effected by persons skilled within  
the art, it is to be understood that this invention is not  
limited to the particular embodiment described by way of  
example hereinabove.

Claims

1. A method of determining a parameter of a sample including the steps of:
  - 5 detecting phase data of a radiation wave field emanating from the sample;
  - combining the phase data with a known parameter of the sample; and
  - determining the required parameter from the phase

10 data and the known parameter of the cell.

  
- 2. The method of claim 1 wherein the parameter comprises the volume of the cell and the method includes:
  - 15 determining the physical thickness of the sample by multiplying phase data contained in the radiation wave field by the wavelength of the radiation and dividing by a prescribed factor of the refractive index of the sample, with the refractive index being the known parameter of the sample; and
  - 20 multiplying the physical thickness by the area of the sample to obtain the volume of the sample.
  
- 3. The method of claim 1 wherein the parameter is the refractive index of the sample and the method includes:
  - 25 confining the sample to a known thickness;
  - detecting the wave field emanating from the sample confined to the known thickness;
  - determining phase data contained in the radiation wave field emanating from the sample;

30 multiplying the phase data by the wavelength of the radiation; and

35 dividing the phase data multiplied by the wavelength, by a prescribed factor multiplied by the physical thickness to which the sample is confined.
  
- 4. An apparatus for determining a parameter of a sample including the steps of:

- 13 -

detecting means for detecting phase data of a radiation wave field emanating from the sample;

processing means for combining the phase data with a known parameter of the sample; and

5 determining the required parameter from the phase data and the known parameter of the cell.

5. The apparatus of claim 4 wherein the parameter comprises the volume of the cell and the processor is for:

10 determining the physical thickness of the sample by multiplying phase data contained in the radiation wave field by the wavelength of the radiation and dividing by a prescribed factor of the refractive index of the sample, with the refractive index being the known parameter of the 15 sample; and

multiplying the physical thickness by the area of the sample to obtain the volume of the sample.

6. The apparatus of claim 4 wherein the parameter is 20 the refractive index of the sample and the apparatus includes:

means for confining the sample to a known thickness;

25 the processor is for detecting the wave field emanating from the sample confined to the known thickness; determining phase data contained in the radiation wave field emanating from the sample;

multiplying the phase data by the wavelength of the radiation; and

30 dividing the phase data multiplied by the wavelength by a prescribed factor and the physical thickness to which the sample is confined.

7. A method of determining the volume of a sample 35 including the steps of:

determining the physical thickness of the sample by multiplying phase data contained in a beam of light

- 14 -

emanating from the sample by the wavelength of the beam of light and dividing by a prescribed factor of the refractive index of the sample; and

5 multiplying the physical thickness by the area of the sample to obtain the volume of the sample.

8. The method of claim 7 wherein the physical thickness is obtained at each pixel of a charge coupled device which detects the light beam emanating from the 10 object and the area value is the area value of the pixel.

9. The method of claim 8 wherein for all pixels which contain the image of the object, the area of the 15 pixel is multiplied by the physical thickness at each pixel and summed in order to provide the volume of the object.

10. The method of claim 9 including mapping on an image of an object the location of the object on the charge coupled device so that all pixels within the mapped area 20 can be determined, determining the thickness of the cell attributed to each of those pixels by multiplying phase data at each pixel by the wavelength of the light and then dividing by  $2\pi$  multiplied by the refractive index of the object, and summing the volume obtained for each of the 25 pixels.

11. An apparatus for determining the volume of a sample, including:

means for detecting light emanating from an 30 object so as to enable phase data relating to the object to be determined; and

35 processing means for determining the phase data from the detected light, and for determining the physical thickness of the object by multiplying the phase data by wavelength of the light and dividing by a factor of the refractive index of the light; and

then calculating the cell volume by an area value

- 15 -

multiplied by the physical thickness.

12. The apparatus of claim 11 wherein the physical thickness is obtained at each pixel of a charge coupled 5 device which detects the light beam emanating from the object and the area value is the area value of the pixel.

13. The apparatus of claim 12 wherein volume is obtained by the sum of the thickness at all pixels which 10 contain the image of the object.

14. The apparatus of claim 13 wherein the processing means maps on an image of an object the location of the object on the charge coupled device so that all pixels 15 within the mapped area can be determined, determining the thickness of the cell at each of those pixels by multiplying phase data at each pixel by the wavelength of the light and then dividing by  $2\pi$  multiplied by the refractive index of the object, and summing the volume 20 obtained for each of the pixels.

15. A method of determining the refractive index of a sample, including the steps of:  
detecting radiation emanating from the sample;  
25 confining the sample to a predetermined thickness;  
determining phase data relating to the radiation wave field emanating from the sample which is confined to the predetermined thickness;  
30 multiplying the phase data by wavelength, and dividing the phase data multiplied by wavelength, by a prescribed factor multiplied by the predetermined thickness to thereby provide the refractive index.  
35 16. The method of claim 15 wherein the sample is confined to the known thickness by squeezing the sample between a pair of plates.

- 16 -

17. The method of claim 15 wherein the predetermined factor is  $2\pi$ .

5 18. An apparatus for determining the refractive index of a sample, including:

detector means for detecting radiation emanating from the sample;

10 means for confining the sample to a predetermined thickness;

processing means for determining phase data relating to the radiation wave field emanating from the sample which is confined to the predetermined thickness; and

15 multiplying the phase data by wavelength and dividing the phase data multiplied by wavelength by a prescribed factor multiplied by the predetermined thickness to thereby provide the refractive index.

20 19. The apparatus of claim 18 wherein the means for confining comprises a pair of plates.

20. The apparatus of claim 18 wherein the predetermined factor is  $2\pi$ .

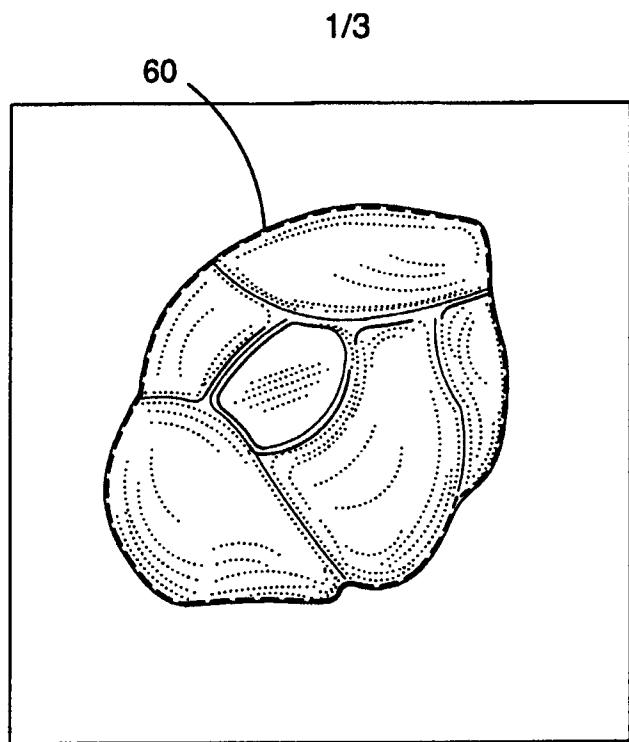


FIGURE 1

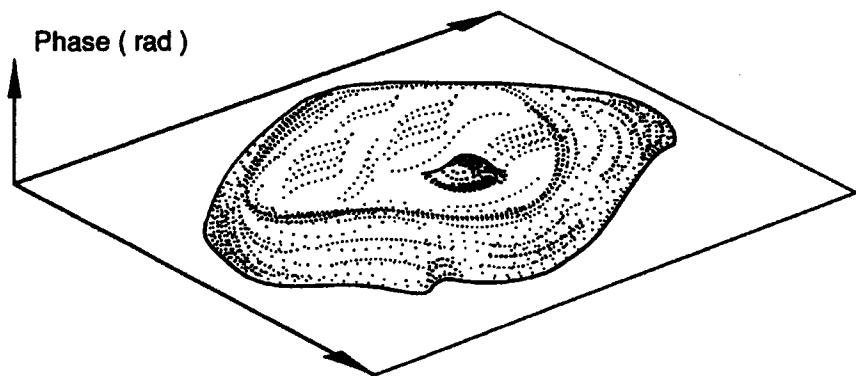


FIGURE 2

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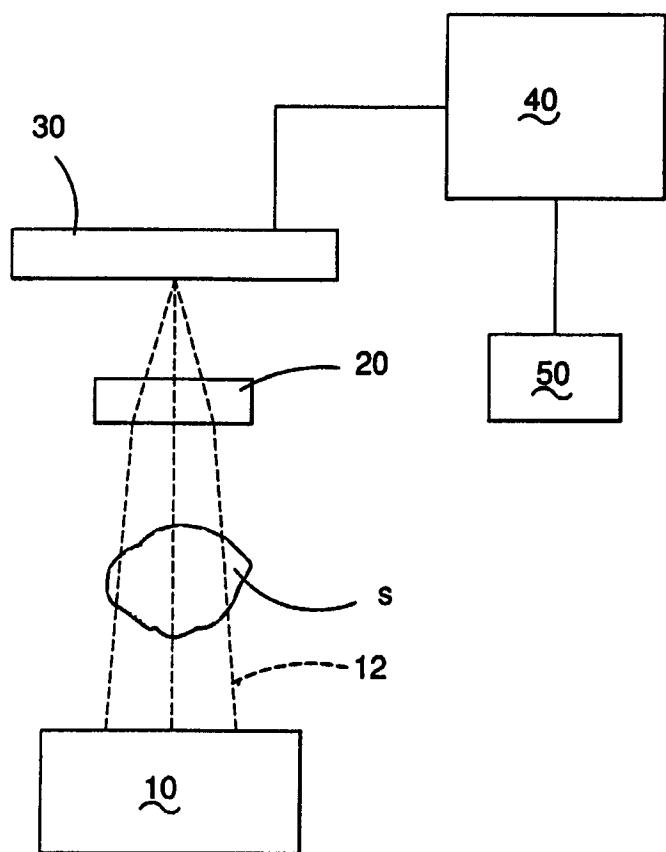


FIGURE 3

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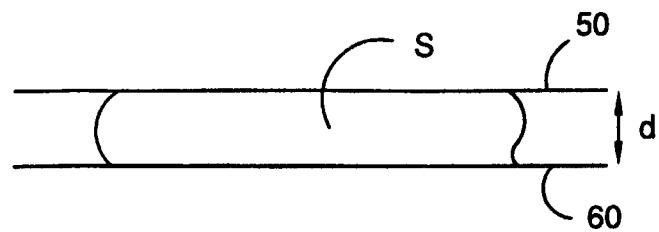


FIGURE 4

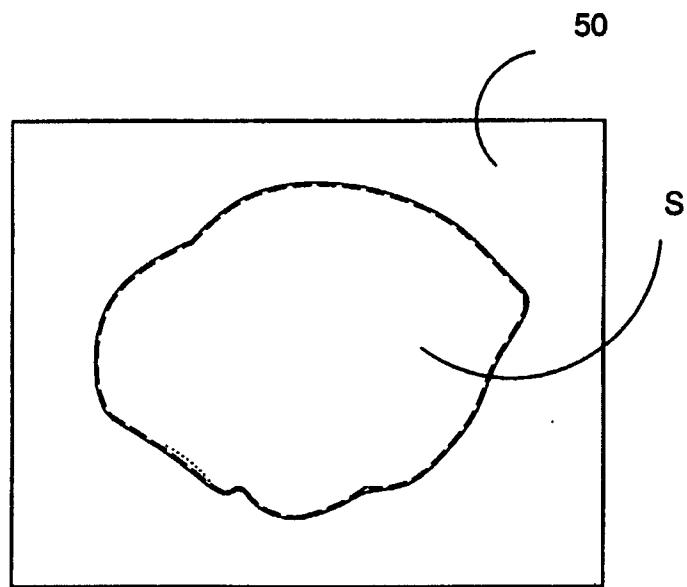


FIGURE 5

## INTERNATIONAL SEARCH REPORT

International application No.  
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<b>A. CLASSIFICATION OF SUBJECT MATTER</b>																						
Int. Cl. <sup>7</sup> : G01N 21/41, 33/483, G01B 11/06, G01J 9/00																						
According to International Patent Classification (IPC) or to both national classification and IPC																						
<b>B. FIELDS SEARCHED</b>																						
Minimum documentation searched (classification system followed by classification symbols)																						
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Electronic data base consulted during the international search (name of data base and, where practicable, search terms used) DWPI, JAPIO   Keywords: optic, light, radiation; phase, optic path length; thickness, G01B 11/06; index, G01N 21/41; cell, biolog, volume; wavefront, wavefield; phase recover, phase retriev, phase reconstruct, G01J 9/00																						
<b>C. DOCUMENTS CONSIDERED TO BE RELEVANT</b>																						
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.																				
X	US 4842408 A (YOSHII et al.) 27 June 1989 Whole document	1-15, 17-18, 20																				
X	The Measurement, Instrumentation, and Sensors Handbook, edited by John G. Webster, Florida: CRC Press, 1999. Section 61.3	1-7, 11, 15, 17-18, 20																				
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**INTERNATIONAL SEARCH REPORT**

Information on patent family members

International application No.

**PCT/AU02/00985**

This Annex lists the known "A" publication level patent family members relating to the patent documents cited in the above-mentioned international search report. The Australian Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent Document Cited in Search Report	Patent Family Member
US 4842408	JP 63179224
	JP 62263428
	JP 62263427

END OF ANNEX